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Renal Sexual Segment of the Ground Skink, *Scincella laterale* (Reptilia, Squamata, Scincidae)

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ABSTRACT Mature squamates possess hypertrophied regions of the distal urinary ducts, the renal sexual segment (RSS). The RSS is believed to provide seminal fluid that mixes with sperm and is released into the female cloaca during coitus. This study is the first to describe ultrastructure of the RSS in a lizard collected throughout the active season. The species examined, *Scincella laterale*, represents the largest family (Scincidae: 1,200 species) of lizards. Although sperm are present in the posterior ductus deferens of male *S. laterale* throughout the year, an annual spermatogenic cycle occurs that results in spermiation in spring, coinciding with maximum development of the RSS. Female *S. laterale* may possess stored sperm in vaginal crypts from March–May and large oviductal eggs April–June. Thus, the correlation between mating and RSS activity observed in other squamates is also found in *S. laterale.* Cytologically, the active RSS consists of columnar cells with numerous apical, electron-dense secretory vacuoles which are released by an apocrine process. The granules stain positively for proteins with bromphenol blue and react with PAS for neutral carbohydrates. After the mating season the RSS undergoes recrudescence and the electron-dense granules are replaced by a mucoid secretion that characterizes more proximal portions of the nephric tubules throughout the year. Little variation in ultrastructure of the RSS occurs between *S. laterale* and *Cnemidophorus lemniscatus* (Teiidae), the only other lizard in which seasonal variation of the RSS has been studied using similar methods. Females exhibit differentiation similar to that of males in the distal urinary tubules, but to a lesser degree. This is only the second such report for female squamates, and the differentiation of the region in females is proposed to result from adrenal androgens. J. Morphol. 266:46–59, 2005.

KEY WORDS: Reptilia; Squamata; renal sexual segment; histology; ultra structure

The renal sexual segment (RSS) is a hypertrophied region of the distal urinary ducts of male snakes and lizards (Sever et al., 2002; Krohmer, 2004). In most snakes the RSS involves only the terminal portion of the distal convoluted tubules (called the preterminal segment by Fox, 1952). In lizards and some snakes the RSS can include the terminal segment of the distal convoluted tubules, postterminal segment, collecting ducts, and/or portions of the ureter (Saint Girons, 1972; Fox, 1977). Studies on lizards to date have reported that the RSS is hypertrophied only during periods of sexual activity and cannot be distinguished from adjacent tubular regions during sexual quiescence (Fox, 1977; Gabri, 1983). In snakes, however, seasonal variation is more variable, but complete regression, as found in lizards, has not been reported (Sever et al., 2002). Although complete involution of the RSS does not occur in snakes, the composition and histological appearance of the secretory product in snakes has a seasonal pattern that correlates with the concentration of plasma androgens (Krohmer, 2004).

The function of the RSS is not clearly understood but its secretions may sustain and activate sperm (Bishop, 1959; Cuellar, 1966), provide courtship pheromones (Volsøe, 1944), form copulatory plugs (Devine, 1975), and/or have other purposes generally associated with seminal fluid (Prasad and Reddy, 1972). Weil (1984) suggested that the RSS secretion of the snake *Nerodia sipedon* has dual functions, one of sperm transport and capacitation in the female reproductive tract in autumn and another related to sexual behavior in the spring.

Numerous histological studies on the RSS of squamates have been done since the first such report by Gampert (1866), and this literature has been reviewed by Saint Girons (1972) and Fox (1977). Only

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*TABLE 1. Specimens examined and RSS diameter**

	Males			Females		
Date	SVL	Dia	SE	SVL	Dia	SE
29 Mar	32.8	0.091	0.0035	44.3	0.055	0.0034
	34.0	0.097	0.0052	45.8	0.070	0.0042
	39.6	0.100	0.0030	47.0	0.090	0.0021
20 Apr	37.9	0.107	0.0055	36.4	0.056	0.0037
	39.7	0.132	0.0042	42.7	0.057	0.0030
	41.7	0.102	0.0066	43.4	0.047	0.0034
11 May	36.3	0.088	0.0029	39.3	0.048	0.0020
	40.2	0.096	0.0043	40.9	0.047	0.0021
	43.8	0.104	0.0030	45.3	0.071	0.0023
19 June	32.1	0.111	0.0043	37.4	0.046	0.0027
	36.1	0.122	0.0044	43.1	0.063	0.0021
	42.4	0.117	0.0063	45.8	0.067	0.0034
				48.9	0.079	0.0035
13 Aug	36.7	0.049	0.0035	40.8	0.044	0.0022
	38.1	0.068	0.0029	41.0	0.041	0.0031
	38.3	0.038	0.0033	42.9	0.043	0.0021
	39.0	0.044	0.0030			
29 Oct	39.0	0.044	0.0027	42.4	0.036	0.0022
	40.3	0.051	0.0023	44.9	0.045	0.0027
	42.7	0.051	0.0023	46.2	0.042	0.0013

*Measurements in mm.

six ultrastructural studies on the RSS have been done, equally divided between snakes and lizards. Furieri and Lanzavecchia (1959) were only concerned with the effects of castration on the endoplasmic reticulum and Golgi apparatus of the sexual segment of the lizard *Lacerta sicula* (Lacertidae). Del Conte and Tamyo (1973) studied the lizard *Cnemidophorous lemniscatus* (Teiidae), in which an area that undergoes some differentiation also occurs in females. Their study was restricted to three adults of each sex collected in September from a locale where continuous sexual activity occurs in *C. lemniscatus*. Gabri (1983) also examined a lacertid, *Podacris taurica*. He examined captive individuals kept under a temperature regime mimicking natural conditions and that were sacrificed from early April to late September. In all of these lizards the sexual segment is limited to the collecting ducts. All of the snakes with RSS examined by electron microscopy are natricine snakes: *Natrix natrix* (Kuhnel and Krisch, 1974), *Seminatrix pygaea* (Sever et al., 2002), and *Nerodia sipedon* (Krohmer, 2004). In these snakes the RSS occurred in portions of the distal convoluted tubules.

The present study concerns seasonal variation at the light and electron-microscopy level of the RSS in the North American skink, *Scincella laterale* (Scincidae). The Scincidae is the largest family of lizards (some 1,200 species), and representatives occur on all continents except Antarctica. Although phylogenetic relationships of lizards are controversial, the Scincidae generally is placed in the same major clade as Teiidae and Lacertidae (Scincomorpha), and this group is considered to be closely allied to snakes (Evans and Barbadillo, 1998). This study provides comparative data that expand our knowledge of the ultrastructural variation of the RSS in male squamates and is the first study to examine wild-caught lizards collected throughout the entire active season. We also provide ultrastructural observations on corresponding areas of the female urinary ducts based on specimens collected concurrently with the male samples.

MATERIALS AND METHODS

Urogenital organs were examined from 20 male and 20 female skinks collected in six sample periods: April 2001, May 2001, October 2001, March 2002, June 2002, and August 2002. All specimens were collected on uncontaminated sites located within the U.S. Department of Energy's Savannah River Site in Aiken County, South Carolina. Specimens were sacrificed on the dates indicated in Table 1, within a week of their capture. Specimens were euthanized by exposure to ether, a procedure approved by the Animal Care and Use Committee of Saint Mary's College, where the specimens were killed. After death, snout–vent length (SVL) was measured from the tip of the snout to the posterior end of the cloacal orifice. Carcasses of all specimens were preserved in 10% neutral buffered formalin and are housed in the research collections at Southeastern Louisiana University, Hammond, LA.

The abdomen was slit midventrally from the cloacal orifice anteriorly to the stomach region. For electron microscopy, preparation of the tissues followed Dawes (1979). The left kidney was removed and fixed in a 1:1 solution of 2.5% glutaraldehyde and 3.7% formaldehyde in cacodylate buffer, pH 7.2. After initial fixation, tissues were rinsed in distilled-deionized water, postfixed in 2% osmium tetroxide, dehydrated through a graded series of ethanol, cleared in propylene oxide, and polymerized in an epoxy resin (Embed 812, Electron Microscopy Sciences, Fort Washington, PA). Plastic sections were cut with an RMC MT7 ultramicrotome (Research and Manufacturing, Tucson, AZ) and DiATOME (Biel, Switzerland) diamond knives. Semithin sections (0.5–1 μm) for light microscopy were placed on microscope slides and stained with Toluidine blue. Ultrathin sections (70 nm) for transmission electron microscopy were collected on uncoated copper grids and stained with solutions of uranyl acetate and lead citrate. Ultrathin sections were viewed with a Hitachi H-300 transmission electron microscope (Nissei Sangyo America, Mountain View, CA).

The right urogenital organs were prepared by the standard paraffin method for light microscopy (Humason, 1979). These reproductive tissues were fixed in 10% neutral buffered formalin, rinsed in water, dehydrated in ethanol, cleared in toluene, and embedded in paraffin. Paraffin sections (10 μm) were cut with a rotary microtome and affixed to albuminized slides. Alternate slides were stained with hematoxylin-eosin (for general cytology), Alcian blue 8GX at pH 2.5 (AB, for primarily carboxylated glycosaminoglycans) followed by the periodic acid-Schiff's procedure (PAS, for neutral carbohydrates and sialic acids), and bromophenol blue (BB, for proteins). Staining procedures followed Kiernan (1990).

For each specimen the largest diameters of 10 tubules from the RSS area were measured from paraffin or plastic sections to the nearest 0.01 with an ocular micrometer inserted in a light microscope. To determine whether the diameter of the RSS differed seasonally between the sexes, we compared the diameter of RSS between the sexes and among collection dates using a two-way analysis of covariance (ANCOVA) with skink SVL as the covariate. Data were first tested for normality and homoscedasticity using Ryan Joiner and Levene tests, respectively (Minitab, v. 13). Although RSS were normally distributed, skink SVL required log 10-transformation to approximate a normal distribution. However, transformation failed to improve observed inequalities in variance between sexes. Therefore, we used a mixed-model approach to ANCOVA (PROC MIXED, SAS, Cary, SC, v. 8.1) with an unspecified covariance matrix structure.

RESULTS

Male Reproductive Cycle

Males collected from March have thick, spermatogenetically active seminiferous epithelia (Fig. 1A). Mature sperm are abundant in the lumina of the seminiferous tubules (Fig. 1A), and sperm are found in the efferent duct system and in all regions of the ductus deferens (Fig. 1B). By June the epithelium of the seminiferous tubules is thinner, but spermiogenesis is still occurring, and the lumina of the tubules contain numerous sperm (Fig. 1C). As in specimens examined from March, April, and May, sperm are present in the vasa efferentia and the entire ductus deferens (Fig. 1D).

In the August sample the seminferous tubules are reduced in diameter and the seminiferous tubules are not spermatogenetically active (Fig. 1E). Sperm are absent from the vasa efferentia and the anterior ductus deferens but still occur in the posterior ductus deferens (Fig. 1F). By October spermatogenesis has been initiated, and mature sperm once more occur in the lumina of seminiferous tubules (Fig. 1G). However, sperm transfer to the efferent ducts has not occurred (Fig. 1H). Sperm remain in the posterior ductus deferens (Fig. 1I).

Renal Sexual Segment

The renal sexual segment of the ground skink includes the posterior convoluted tubules and collecting ducts of the nephrons as well as the ureter (Fig. 2). Although females were significantly larger than males (SVL = 43 ± 0.7 mm vs. 38 ± 0.7 mm; $P < 0.05$), the RSS were larger in males than in females during the reproductive season (Fig. 3). After controlling for the effects of body size (log SVL: $F = 6.08$, $P = 0.02$), statistical comparison revealed that the diameter of the RSS differed between sexes but that this difference was dependent on season (Sex × Time interaction: $F = 7.97$; $P < 0.001$). The RSS is highly hypertrophied and actively secretory in males collected from March, April, May, and June (Table 1; Figs. 2A–C, 3–6). The mean monthly RSS diameters for males from March–June are 0.09–0.12 mm, and these samples form a homogenous subset (Fig. 3). The secretory product consists of eosinophilic granules in the RSS epithelium that also stain positively with bromphenol blue (BB) for proteins and periodic acid and Schiff's reagent (PAS), indicating neutral carbohydrates. The granules do not react with Alcian blue 8GX (AB) at pH 2.5, thus indicating the absence of primarily carboxylated glycosaminoglycans, although more proximal tubules of the nephron are AB+.

Monthly mean diameters of females from all samples and males collected from August and October varied from 0.04–0.07 mm in diameter. The mean RSS diameter (0.07) of the March sample of females was larger than in late season female samples, indicating some seasonal hypertrophy in females, but the differentiation is much less than in active males (Figs. 2D, 3, 7). Generally, in males from August and September and in females from all samples, tubules in the area of the RSS have only a slight PAS+ reaction around the lumen, and the BB+ staining of the cytoplasm is not uniform. The secretory product of females and males from the late summer and fall is in the form of an AB+ mucoid secretion (Figs. 8, 9) that is indistinguishable between males and females and between proximal and distal nephric tubules.

Ultrastructure

March–June. In the spring and early summer the epithelium of the male renal sexual segment consists of columnar cells with basal, euchromatic nuclei with prominent, usually eccentric nucleoli (Figs. 4–6). In March extensive supranuclear Golgi complexes occur and rough endoplasmic reticulum is abundant throughout the cytoplasm (Fig. 4A,B). Secretory granules in various stages of maturity occur, with condensing vacuoles associated with the trans face of the Golgi complexes (Fig. 4B) and electron-dense, spherical secretory vacuoles (1–3 μm dia.) are abundant in the apical cytoplasm (Fig. 4C,D). Secretory vacuoles appear uniformly dense and most of them possess an outer, often irregular collar. This collar is composed of material of lesser density than the secretory vacuoles, but greater density than surrounding cytoplasm (Fig. 4C,D). Cell junctions are obscure between cells except for terminal tight junctions (Fig. 4D). Secretory vacuoles appear to be released independently, with some peripheral cytoplasm, into the lumen. Thus, secretion is an apocrine process. Dissolution of the vacuoles in the

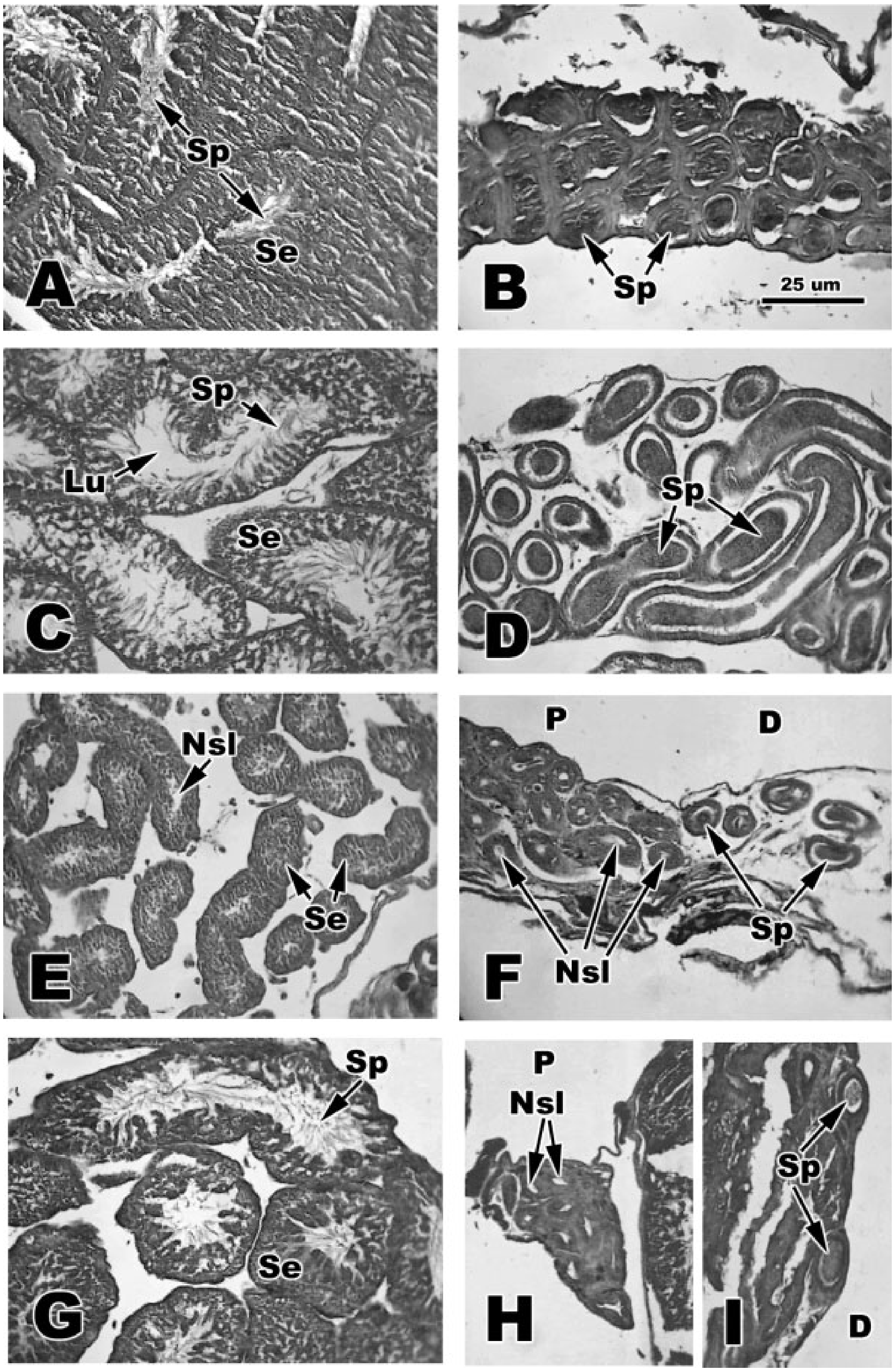

Fig. 1. *Scincella laterale.* Reproductive cycle of males. Testis (**A**) and ductus deferens (**B**) of a 32.8 mm SVL specimen sacrificed 29 March. Testis (**C**) and ductus deferens (**D**) of a 32.1 mm SVL specimen sacrificed 19 June. Testis (**E**) and ductus deferens (**F**) of a 39.0 mm SVL specimen sacrificed 13 August. Testis (**G**) and proximal ductus deferens (**H**) and distal ductus deferens (**I**) of a 42.7 mm SVL specimen sacrificed 29 October. D, distal ductus deferens; Lu, lumen; Nsl, no sperm in lumen; P, proximal ductus deferens; Se, seminiferous epithelium; Sp, sperm.

lumen results in a secretory product less dense than the central core (Fig. 4C,D).

In specimens examined from April–June mature secretory vacuoles appear to occupy more of the cytoplasm and Golgi complexes are not numerous (Fig. 5A). As in the March sample, intercellular membranes are obscure apically except for the terminal tight junctions. Although the synthesis of additional

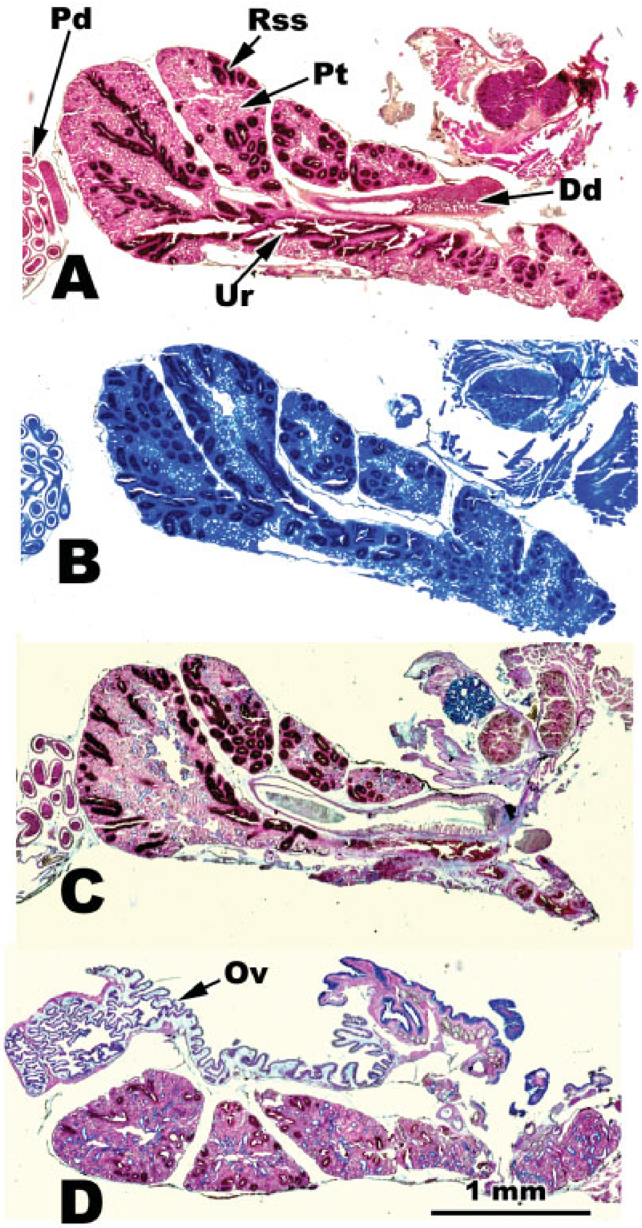

Fig. 2. *Scincella laterale.* Renal sexual segment as seen in sagittal paraffin sections of a male 36.1 mm SVL (**A–C**) and of a 48.9 mm SVL female (**D**) sacrificed 19 June. **A:** Hematoxylin-eosin. **B:** Bromphenol blue. **C,D:** Alcian blue at pH 2.5. Dd, distal ductus deferens; Ov, oviduct; Pd, proximal ductus deferens, Pt, proximal tubules, Rss, renal sexual segment; Ur, ureter.

vacuoles has slowed from March, production of additional secretory vacuoles clearly continues through June (Figs. 5, 6).

In addition, ultrastructural examination of tissue from specimens collected in May and June indicate areas with an abundance of distended cisternae of rough endoplasmic reticulum (RER) among the secretory vacuoles (Fig. 6C,D). Product synthesis also occurs in these areas, with numerous supranuclear Golgi complexes and condensing vacuoles in various degrees of maturation (Fig. 6C,D). The contrast between the regions with smaller, darker profiles of RER (Fig. 4) to areas with distended RER cisternae (Fig. 5) is quite striking, and suggests that different mechanisms of product synthesis and synthesis of different products occur.

The RSS area in females appeared most highly developed in June specimens; the ultrastructure of this region in one such female is illustrated in Figure 7. Mature granules are not as numerous as in males, but occupy the apical third of the epithelial cells (Fig. 7A). The basal nuclei in the epithelial cells seem more heterochromatic (Fig. 7B) than those of males. Secretory product is actively being synthesized, however, as evidenced by condensing vacuoles in various degrees of formation associated with Golgi complexes, narrow profiles of RER, and dark, elongate mitochondria (Fig. 7C). Once again, the divisions between adjacent cells are not apparent apically except for terminal tight junctions (Fig. 7D).

August. In the specimens from August the RSS has become nearly indistinguishable from other tubular portions of the nephron. Cells in the tubules of the RSS area are AB+ and do not react strongly with PAS or BB, and the RSS are no longer larger in diameter than other tubules in the nephron. At the ultrastructure level, the only way to distinguish the RSS from more proximal tubules of the nephron is due to the presence of a few electron-dense secretory vacuoles remaining in the apical cytoplasm of scattered cells of the RSS (Fig. 8A,B). The epithelial cells have become more cuboidal and the nuclei are more irregular and heterochromatic (Fig. 8A–C). Synthetic organelles still occur (Fig. 8B,C), but these seem involved in the production of an acidic mucoid secretion (Fig. 8C,D) as determined by the AB+ reaction of the apical cytoplasm. Electron-lucent vacuoles are common, especially basally (Fig. 8C). The intercellular canaliculi are more conspicuous and wider than in the spring and early summer samples, and these plasma membranes between the cells are often labyrinthine (Fig. 8D).

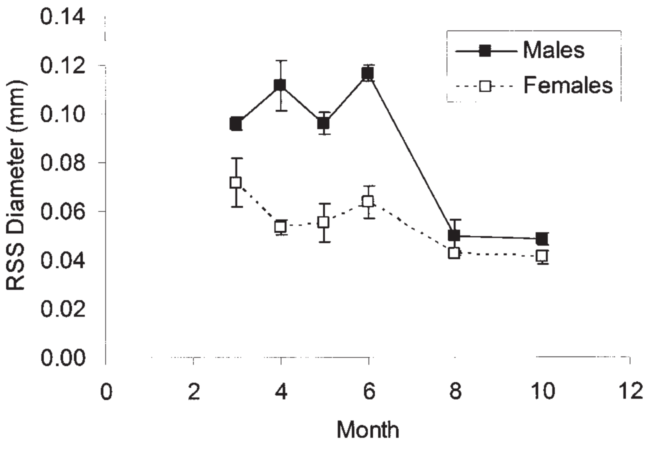

Fig. 3. *Scincella laterale.* Mean monthly diameter of the renal sexual segment (RSS) from males and females. Data are presented as mean diameter ± 1 SE.

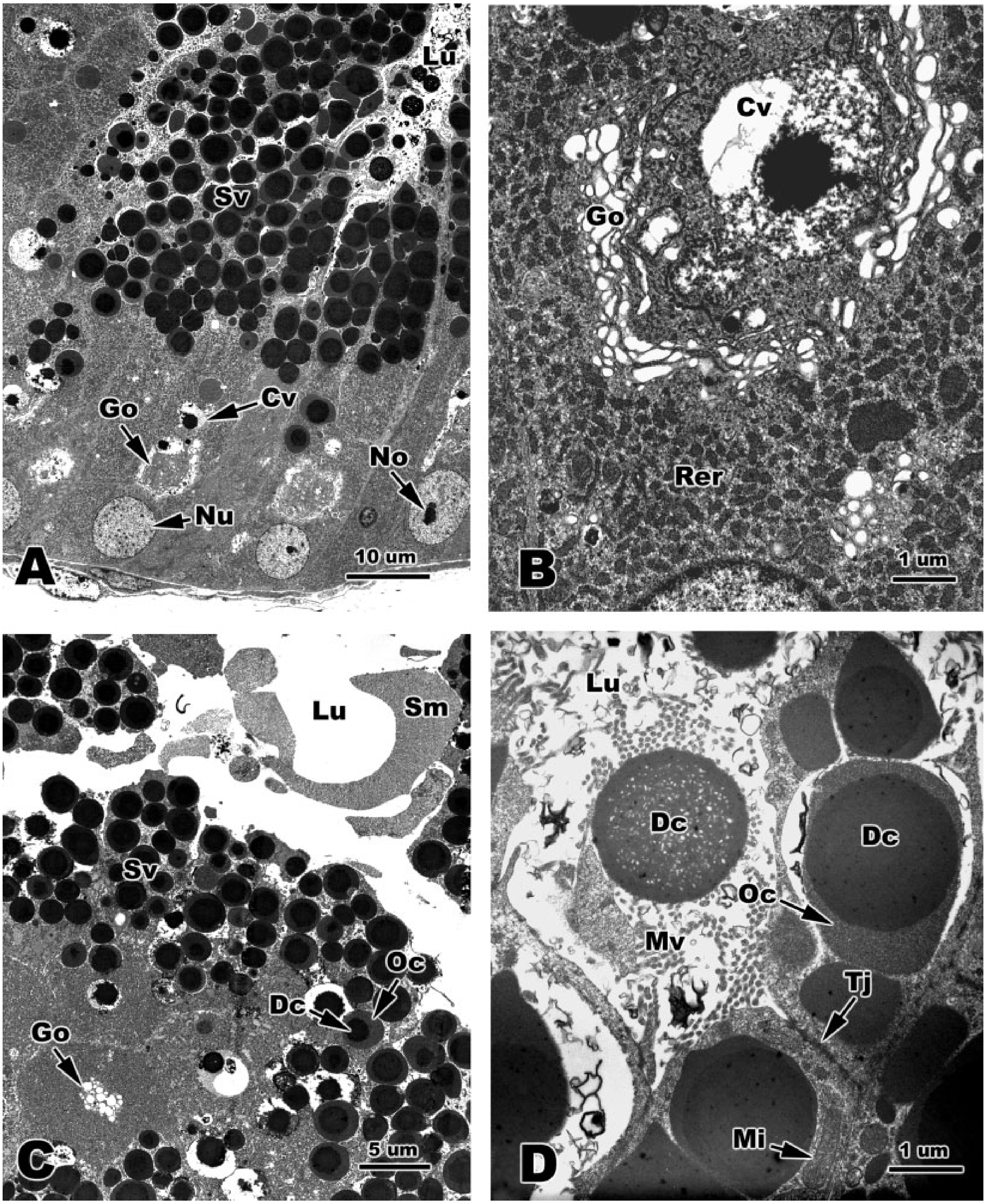

Fig. 4. *Scincella laterale.* Transmission electron micrographs through the renal sexual segment in the kidney of a 34.0 mm SVL male sacrificed 29 March. **A:** Section through several epithelial cells from basal nuclei to the lumen. **B:** Supranuclear cytoplasm showing details of the synthetic organelles. **C:** Luminal border. **D:** Release and dissolution of secretory vacuoles. Cv, condensing vacuoles; Dc, dense core; Go, Golgi complex; Lu, lumen; Mi, mitochondria; Mv, microvilli; No, nucleolus; Nu, nucleus; Oc, outer collar; Rer, rough endoplasmic reticulum; Tj, tight junction.

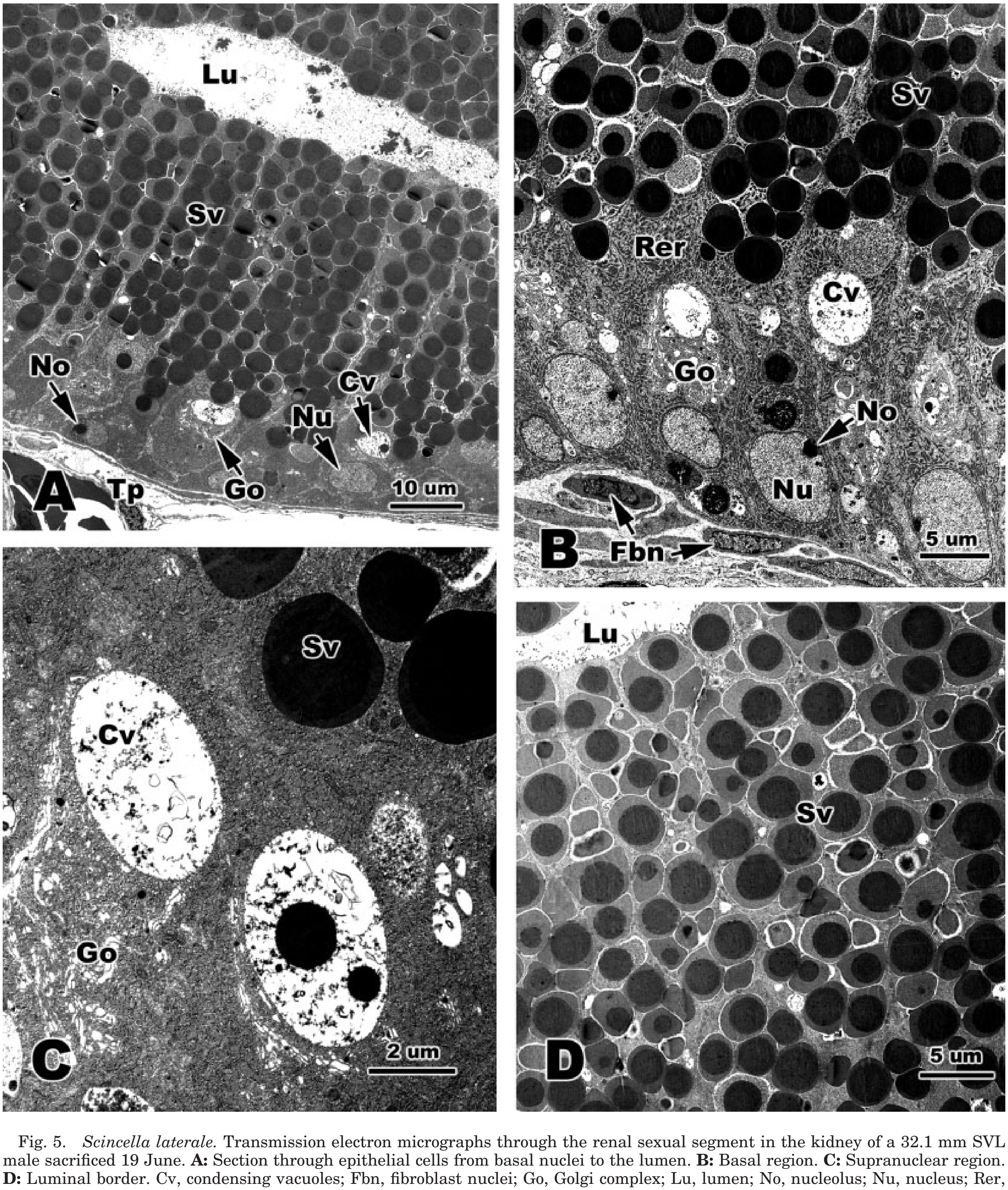

Fig. 5. *Scincella laterale.* Transmission electron micrographs through the renal sexual segment in the kidney of a 32.1 mm SVL male sacrificed 19 June. **A:** Section through epithelial cells from basal nuclei to the lumen. **B:** Basal region. **C:** Supranuclear region. **D:** Luminal border. Cv, condensing vacuoles; Fbn, fibroblast nuclei; Go, Golgi complex; Lu, lumen; No, nucleolus; Nu, nucleus; Rer, rough endoplasmic reticulum; Tp, tunica propria.

October. In the October sample, the only way one can distinguish which tubules compose the RSS within the kidney is by location, i.e., the distal convoluted tubules and collecting ducts are peripheral to the central mass of the kidney where proximal tubules and glomeruli are located. In tangential sections that do not include the lumen, the RSS tubules appear as inactive cords of cells (Fig. 9A). When the sections include the lumen, the epithelial cells are cuboidal, as in the August sample, except that the cytoplasm is

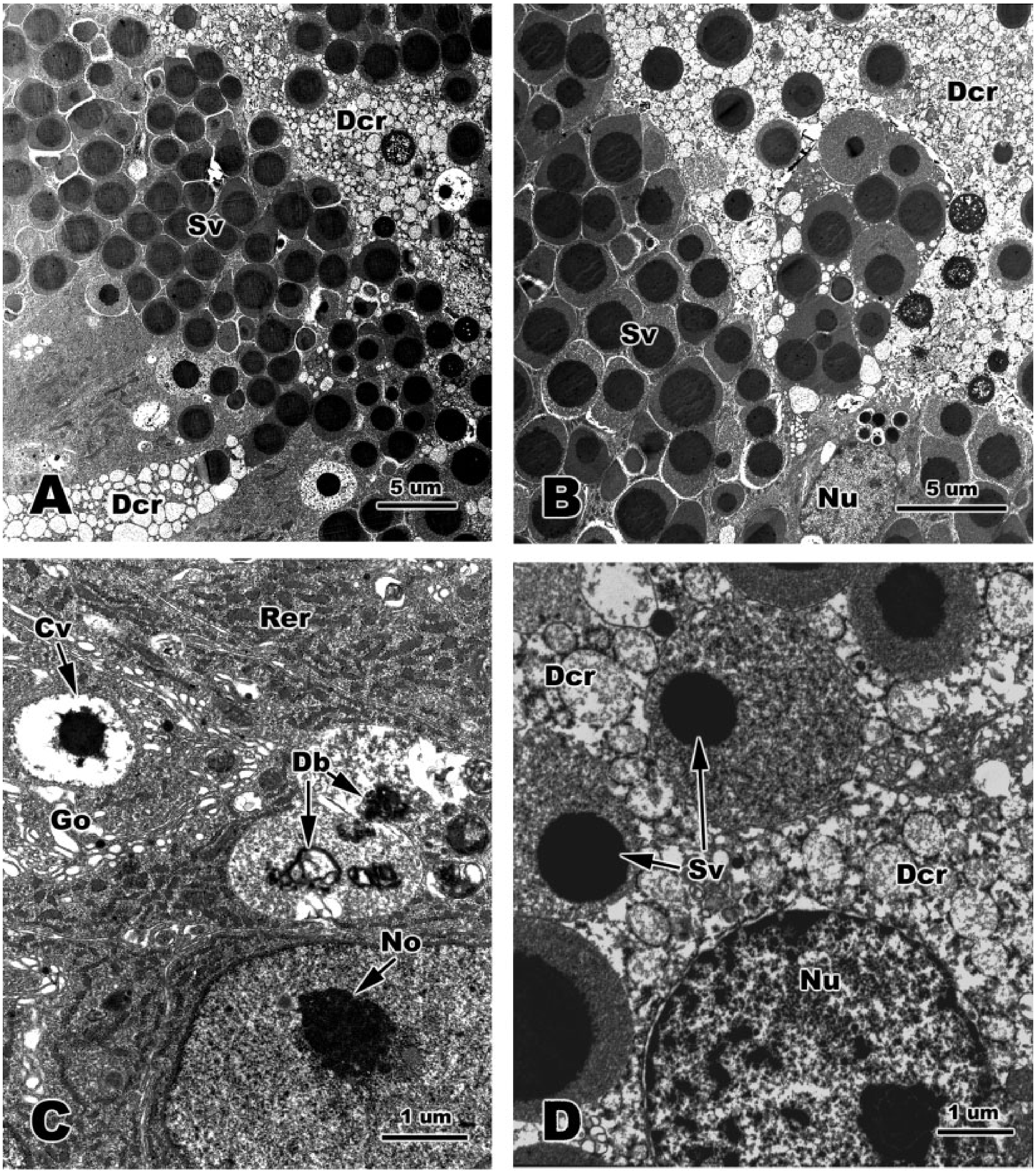

Fig. 6. *Scincella laterale.* Transmission electron-micrographs through the renal sexual segment in the kidney of the same specimen used in Figure 5. **A:** Section through several epithelial cells possessing distended cisternae of endoplasmic reticulum. **B:** Supranuclear cytoplasm. **C:** Synthetic organelles in supranuclear area. **D:** Supranuclear area with mature secretory vacuoles and distended cisternae. Cv, condensing vacuoles; Db, dense bodies; Dcr, distended cisternae of endoplasmic reticulum; Go, Golgi complex; No, nucleolus; Nu, nucleus; Rer, rough endoplasmic reticulum.

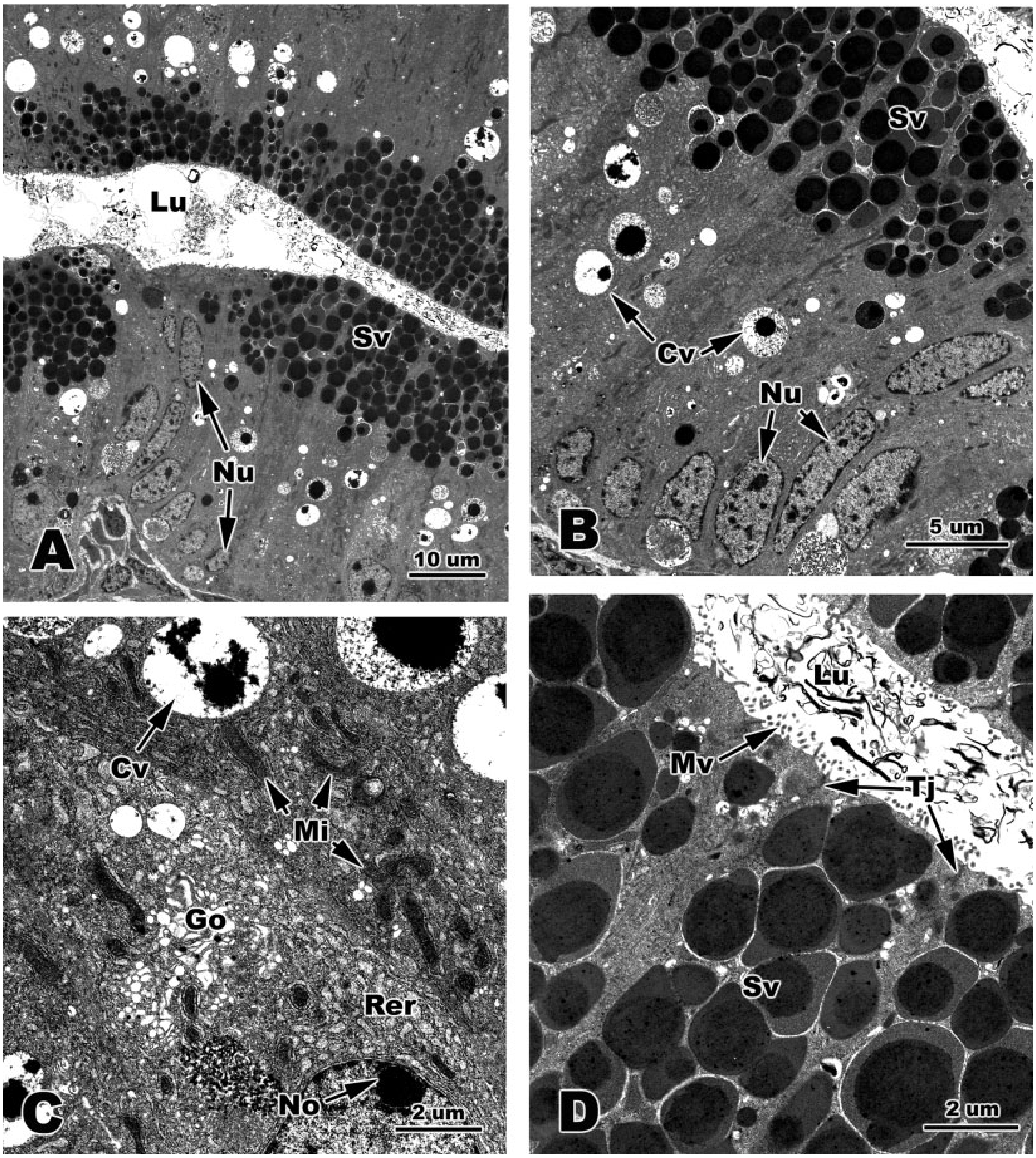

Fig. 7. *Scincella laterale.* Transmission electron micrographs through the renal sexual segment in the kidney of a 48.9 mm SVL female sacrificed 19 June. **A:** Section through epithelial cells from basal region to the lumen. **B:** Several epithelial cells. **C:** Supranuclear cytoplasm with synthetic organelles. **D:** Luminal border. Cv, condensing vacuoles; Go, Golgi complex; Lu, lumen; Mi, mitochondria; Mv, microvilli; No, nucleolus; Nu, nucleus; Rer, rough endoplasmic reticulum; Sv, secretory vacuoles; Tj, tight junction.

even more reduced in October (Fig. 9B). Again, mucoid secretory vacuoles cluster along the luminal border of many epithelial cells, and organelles such as Golgi complexes, RER, and mitochondria involved in product synthesis occur in the cytoplasm (Fig. 9C,D).

DISCUSSION

Seasonal Variation

Hypertrophy and recrudescence of the RSS are synchronous with androgen secretion and spermat-

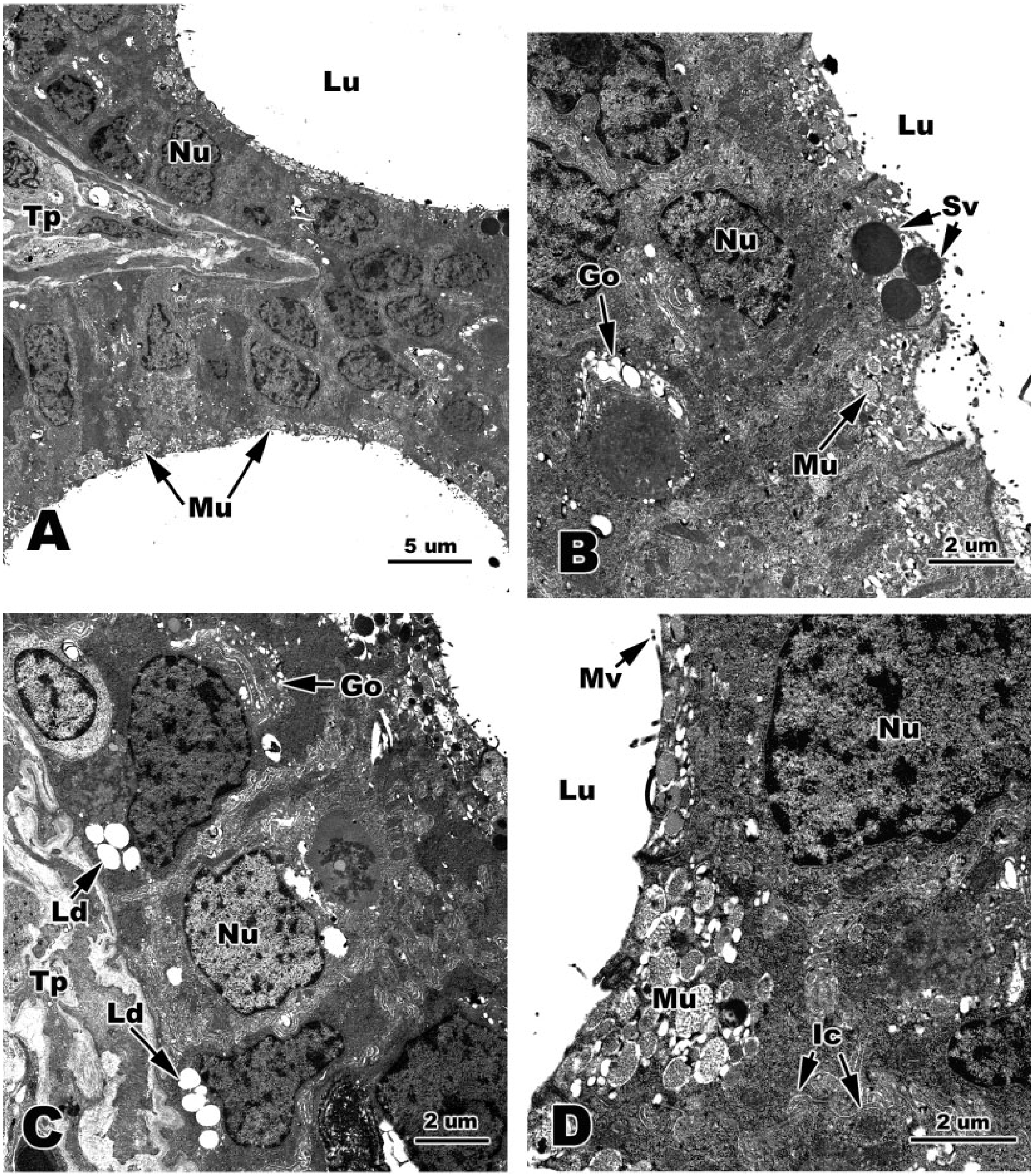

Fig. 8. *Scincella laterale.* Transmission electron micrographs through the renal sexual segment in the kidney of a 39.0 mm SVL male sacrificed 13 August. **A:** Section through two adjacent tubules. **B:** Epithelial cells from basal region to the lumen. **C:** Epithelium with basal lipid droplets. **D:** Epithelium with apical mucus vacuoles. Go, Golgi complex; Ic, intercellular canaliculus; Ld, lipid droplet; Lu, lumen; Mu, mucus vacuoles; Mv, microvilli; Nu, nucleus; Sv, secretory vacuoles; Tp, tunica propria.

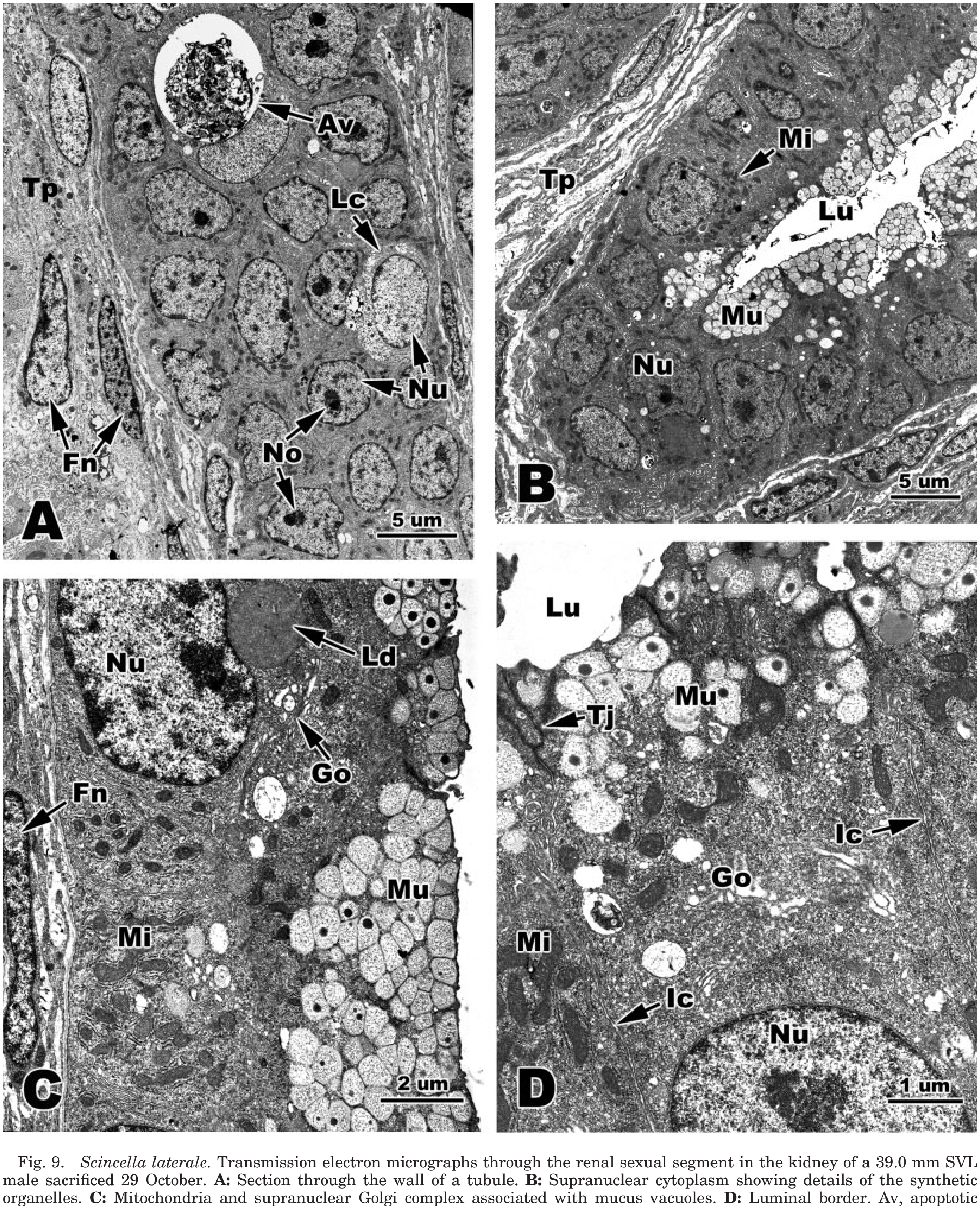

Fig. 9. *Scincella laterale.* Transmission electron micrographs through the renal sexual segment in the kidney of a 39.0 mm SVL male sacrificed 29 October. **A:** Section through the wall of a tubule. **B:** Supranuclear cytoplasm showing details of the synthetic organelles. **C:** Mitochondria and supranuclear Golgi complex associated with mucus vacuoles. **D:** Luminal border. Av, apoptotic vacuole; Fn, fibroblast nucleus; Go, Golgi complex; Ic, intercellular canaliculus; Lc, light cell; Ld, lipid droplet; Lu, lumen; Mi, mitochondria; Mu, mucus vacuoles; Nu, nucleus; Tj, tight junction; Tp, tunica propria.

ogenic activity (e.g., Bishop, 1959; Sanyal and Prasad, 1966; Prasad and Sanyal, 1969; Weil, 1984; Krohmer, 2004). Even in snakes, which show modest or no regression of the RSS during sexual quiescence, variations in the appearance and makeup of the granules provide an identifiable and quantifiable seasonal pattern that can be correlated with the concentration of plasma androgens (Krohmer, 2004).

Although sperm are present in the posterior ductus deferens of male *Scincella laterale* throughout the year, an annual spermatogenic cycle results in spermiation in spring, coinciding with maximum development of the RSS. Female *S. laterale* from this locality may possess stored sperm in vaginal crypts during March, April, and May and large oviductal eggs in April, May, and June (Sever and Hopkins, 2004). Thus, the correlation between mating and RSS activity observed in other squamates is also found in *S. laterale.*

Del Conte and Tamayo (1973) found that females of the teiid lizard *Cnemidophorus lemniscatus* also possess a differentiated area of the collecting duct similar to that of the RSS of males. However, they reported that the cells are smaller and the region is shorter and sometimes discontinuous, although the secretory product shows the same morphological and histochemical properties. Our discovery of a similar situation in females of *Scincella laterale* is only the second report of some degree of differentiation similar to that of males in the distal urinary tubules of female squamates.

Prasad and Sanyal (1969) demonstrated that anabolic and androgenic steroids stimulated development of the RSS in males and females of the gecko, *Hemidactylus flaviviridis,* and that estrogen and progesterone had no stimulatory effect. Billy and Crews (1986) found that administration of testosterone to the parthenogenetic (all phenotypically female) teiid lizard *Cnemidophorous uniparens* resulted in development of the RSS. Thus, the natural occurrence of some hypertrophy of the distal urinary ducts in females of *C. lemniscatus* and *Scincella laterale* is probably due to the presence of plasma androgens. We suggest that the source of these androgens may be the adrenal cortex. In the lizards *Tupinambis* and *Lacerta viridis*, the adrenal gland is capable of testosterone synthesis (Mendi and Sandor, 1974), and Krohmer (2004) suggested that the adrenal gland is a secondary source of androgens in the snake *Natrix sipedon*.

Perhaps the assumption that differentiation of an RSS occurs only in males has led investigators to ignore females in their studies. Therefore, female lizards of other species should be examined more closely to see if some differentiation of the urinary ducts occurs similar to what has been described for *Cnemidophorus lemniscatus* and *Scincella laterale*, and studies to determine if spikes in androgen levels correlate with occurrence of RSS-like areas in females are needed.

Characteristics of the Secretion

Much literature exists on the nature of the secretion of the active RSS and this material has been reviewed extensively in recent articles by Sever et al. (2002) and Krohmer (2004). The secretory vacuoles (also called secretory granules by some authors) at the ultrastructural level have invariably been described as electron dense, and are very similar in appearance to the proteinaceous zymogen granules associated with the chief cells of the stomach or pancreatic acinar glands (Kierszenbaum, 2002). Mature vacuoles of *Cnemidophorus lemniscatus* illustrated by Del Conte and Tamayo (1973) show a lighter collar around a denser core, similar to the condition reported herein for *Scincella laterale*. The PAS+ and BB+ reactions found in *S. laterale* have also been reported for other squamates, such as the snake *Seminatrix pygaea* (Sever et al., 2002). Other studies have indicated that granules may contain a complex of lipids, glycogen, mucopolysaccharides, mucoproteins, and phosphatases, and that the chemical composition may change during the active season (Weil and Aldridge, 1985; Krohmer, 2004).

Depeiges and Dufaure (1980) characterized the epididymal secretory vacuoles of the lacertid lizard *Lacerta vivipara* as being composed of a "dense central core and a peripheral vacuole." This cytology is very similar to that of the secretory vacuoles of the RSS of *Scincella laterale*, in which we found a dense core surrounded by a more electron-lucent collar. Depeiges and Dufaure (1980) found that the core is composed of a protein, or a tight group of proteins, of high molecular weight (about 70,000). The peripheral vacuole contains polysaccharides, possibly a glycoprotein. The epididymis of squamates is the only source of seminal fluid other than the RSS. The similarity in ultrastructure between the epididymal and RSS secretory vacuoles indicates that a similar product may be produced.

The ultrastructural studies on the snakes *Natrix natrix* (Kuhnel and Krisch, 1974) and *Seminatrix pygaea* (Sever et al., 2002) found evidence of an apocrine mode of secretion. However, in the snake *Nerodia sipedon*, Krohmer (2004) proposed that the mature vacuoles are converted from solid to diffuse, with substances then transported through the cytoplasm to the lumen, where they are released by a merocrine process or diffusion. In the lizard *Cnemidophorus lemniscatus*, Del Conte and Tamayo (1973) proposed that the secretory mechanism in areas with the most vacuoles (filling nearly the entire cells) is holocrine, because cells are found in the lumen that retain their nuclei and the general arrangement of their cytoplasmic organelles, but have no plasma membrane. In regions where mature vacuoles are found only in the apical portions of the cells, however, Del Conte and Tamayo (1973) reported a merocrine mode of release. In *Podacris taurica*, Gabri (1983) found that during release of the secretory product the lumen contains vesicles

TABLE 2. Comparisons among lizards on which ultrastructure of the RSS was studied

Season	Character	*Cnemidophorus lemniscatus*	*Podacris taurica*	*Scincella laterale*
Sexually active				
	Location of RSS	Collecting ducts	Collecting ducts	Distal convoluted tubules, collecting ducts, ureter
	Epithelium	Columnar	Columnar	Columnar
	Nuclei	Basal	Basal, euchromatic	Basal, euchromatic
	Intercellular canaliculi	Distinct	Distinct, wide basally	Indistinct
	Golgi complexes	Prominent	Prominent	Prominent
	Distended Rer	Yes	Not mentioned	Yes
	Apical vacuoles	Electron-dense	Fibrous	Electron-dense
	Histochemistry	PAS+, AB−	Not mentioned	PAS+, AB−, BB+
	Secretion	Holocrine	Apocrine	Apocrine
	Occurrence in female	Similar but less developed	Absent	Similar but less developed
Sexually inactive		Not studied		
	Epithelium		Cuboidal	Cuboidal
	Light/dark cells		Present	Present
	Nuclei		Elongate, basal	Irregular, heterochromatic
	Intercellular canaliculi		Wide, interdigitating	Narrow, straight/tortuous
	Golgi complexes		Prominent	Prominent
	Distended RER		Yes	No
	Apical vacuoles		Mucoid	Mucoid
	Histochemistry		Not mentioned	Slight PAS+, BB+
	Lipid droplets		Not mentioned	Yes

and cytoplasmic components derived from the cells, which indicates an apocrine process. We propose an apocrine process for *Scincella laterale* as well; we clearly illustrate whole vacuoles and surrounding cytoplasm undergoing dissolution in the lumen (Fig. 4).

Comparisons in Location and Ultrastructure

Major ultrastructural characteristics of the three species of lizards that have been studied by this method are summarized in Table 2. In addition, Furieri and Lanzavecchia (1959) conducted an ultrastructural study on the RSS of *Lacerta sicula* in which they demonstrated that the cytomembranes of the endoplasmic reticulum and Golgi vesicles lose their characteristic structure and become unrecognizable after castration.

Del Conte and Tamayo (1973) examined only three males and three females of *Cnemidophorus lemniscatus*. These animals were collected in September from a locality in Venezuela where the species exhibits "continuous sexual activity" according to the authors. It would be interesting to discover whether cyclic changes in the RSS are absent due to the lack of a definite mating season. The RSS of the males consists of a first portion comprising the middle area of the collecting duct, in which secretory vacuoles were most abundant. A second area, with fewer secretory vacuoles, is composed of the final portion of the collecting duct and other ducts converging to the ureter. The area that differentiates on a lesser scale in females is equivalent to the first portion in males.

The *Podacris taurica* studied by Gabri (1983) were kept in captivity with temperature regimes apparently mimicking natural conditions (Balkans, Crimean Peninsula). Two males and one female were killed in April, May, mid-July, and late September. In males the RSS consists of a region of the collecting duct directly following the distal tubule and a mucus-secreting region forms the rest of the collecting tubule. In females no RSS is present, and the collecting tubule consists of mucus-secreting cells indistinguishable from those of the male. Gabri found that when males are "spermatogenically active" in spring the RSS secretes a protein, possibly a nutrient for the sperm. Maximum activity is in May, when the apical region of the epithelium is filled with clusters of large vacuoles with "fibrous" contents, although his illustrations indicates that these vacuoles have a high degree of electron density. In July, few apical vacuoles remain, and in September the cells in this region revert to mucus secretion and are similar to other segments of the nephric tubules.

As shown in Table 2, few differences occur among the three species of lizards in which the RSS has been studied by transmission electron microscopy. The differences and similarities in secretory mode have been discussed under the previous heading. The snakes in which the RSS has been studied by ultrastructure are also overall very similar to the lizards, except that the snakes do not show the dramatic seasonal changes in secretory activity observed in *Cnemidophorus lemniscatus* and *Scincella laterale*.

The RSS is more extensive in *Scincella laterale* than in the other lizards. This character shows much variation among lizards, although in snakes the RSS seems invariably limited to the medial region of the distal convoluted tubule (Fox, 1977). However, our knowledge of variation in the location of the RSS within the kidney is based on relatively few species. Examination of more species within the Scincidae and within other families of squamates will be required to determine if a phylogenetic trend occurs in this aspect of RSS structure.

Another difference among the lizards studied by electron microscopy relates to the appearance of the plasma membranes separating epithelial cells. In *Cnemidophorus lemniscatus* and *Podacris taurica* the intercellular canaliculi are distinct, and are especially widened in *P. taurica* during the inactive phase. In *Scincella laterale*, however, the intercellular canaliculi are indistinct, especially in the active phase of the cells, and the epithelium appears almost syncytial.

The widened cisternae of RER of the active phase of the RSS in *Cnemidophorus lemniscatus* and *Scincella laterale*, which persist in *C. lemniscatus* even in the inactive phase, resemble the cisternae of the thyroid gland that are distended during the synthesis of thyroglobulin precursors, which are subsequently glycosylated in the Golgi apparatus (Kierszenbaum, 2002). A similar pathway may occur in synthesis of the mature secretory vacuoles of the RSS in these lizards, i.e., a protein component produced by the RER is combined with a carbohydrate moiety produced by the Golgi complex.

As stated by Sever et al. (2002), the presence of the RSS is a synapomorphy for squamate reptiles, and we have much yet to learn about the diversity of the character within the 7,700+ species (European Molecular Biology Laboratory Database, 2004) that comprise this group. However, the RSS is essential to mating activity, and one cannot truly understand the reproductive biology of squamates without considering the secretory cycle of the RSS.

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